CLAIMS

1. A method for separating a DNA molecule from a mixture of DNA molecules, which method comprises:

(i) amplifying the DNA molecules in the mixture;

(ii) hybridising single strands of the amplified DNA molecules with a complementary strand of a reference DNA molecule so as to form duplexes; and

(iii) separating the duplexes.

2. A method according to claim 1 which comprises

(i) amplifying the DNA molecules in the mixture employing a pair of primers in which one of the primers carries a ligand, so as to produce an amplified mixture of double-stranded DNA molecules in which one of the strands carries a ligand;

(ii) contacting the amplified mixture of double-stranded DNA molecules with a receptor on a solid support under conditions such that the ligand binds to the receptor;

(iii) separating the mixture of double-stranded DNA molecules into single-strands and removing the strands that are not bound to the support by the ligand;

(iv) recovering the remaining strands from the support;

(v) mixing the recovered strands with a

complementary strand of a reference DNA molecule so as to form duplexes; and

(vi) separating the duplexes.

3. A method according to claim 2 wherein the complementary strand of the reference DNA molecule is provided by

(i) amplifying the reference DNA molecule employing a pair of primers in which one of the primers carries a ligand, so as to produce amplified double-stranded reference DNA molecule in which one of the strands carries a ligand;

(ii) contacting the double-stranded reference DNA molecule with a receptor on a solid support under conditions such that the ligand binds to the receptor;

(iii) separating the double-stranded reference DNA molecule into single-strands and removing the strand that is not bound to the support by the ligand; and

(iv) recovering the remaining strand from the support.

4. A method according to claim 2, modified by recovering the strands that are not bound to the support instead of the strands that are bound to the support, and mixing these recovered strands with a complementary strand of a reference DNA molecule in step (v).

5. A method according to claim 3, modified by recovering the strand of the reference DNA molecule that is not bound to the support instead of the strand that is bound to the

support, and using this recovered strand in step (v) of claim 2.

6. A method according to claim 1 which comprises

(i) amplifying the DNA molecules in the mixture employing a pair of primers in which one of the primers carries a high molecular weight molecule, so as to produce an amplified mixture of double-stranded DNA molecules in which one of the strands carries a high molecular weight molecule;

(ii) separating the mixture of double-stranded DNA molecules into single strands;

(iii) mixing the single strands with a complementary strand of a reference DNA molecule so as to form duplexes; and

(iv) separating the duplexes.

7. A method according to claim 6 wherein the complementary strand of the reference DNA molecule is provided by

(i) amplifying the reference DNA molecule employing a pair of primers in which one of the primers carries a high molecular weight molecule, so as to produce an amplified double-stranded reference DNA molecule in which one of the strands carries a high molecular weight molecule; and

(ii) separating the double-stranded reference DNA molecule into single strands.

8. A method according to claim 1 which comprises

(i) amplifying a single strand of each of the DNA molecules in the mixture;

(ii) mixing the amplified single strands with a complementary strand of a reference DNA molecule so as to form duplexes; and

(iii) separating the duplexes.

9. A method according to claim 8 wherein the complementary strand of the reference DNA molecule is provided by amplifying a single strand of the reference DNA molecule.

10. A method according to any one of the preceding claims wherein the reference DNA molecule has a known sequence.

11. A method according to any one of the preceding claims wherein the duplexes are separated by gel electrophoresis.

12. A method according to claim 11 wherein the electrophoresis is performed under denaturing conditions.

13. A method for identifying a DNA molecule in a mixture of DNA molecules, which method comprises separating the DNA molecules by a method as defined in claim 11 or 12, and comparing the positions of the separated duplexes on the gel with the position of a control DNA molecule.

14. A method for identifying a DNA molecule in a mixture of DNA molecules, which method comprises separating the DNA molecules by a method as defined in any one of claims 1 to 12 and sequencing each of the separated DNA molecules, carrying out sequence specific primer (SSP) amplification analysis or carrying out sequence specific oligonucleotide (SSO)

analysis.

15. A method according to any one of the preceding claims wherein the mixture of DNA molecules is a mixture of alleles of a polyallelic gene.

16. A method according to claim 15 wherein a reference allele is used which has the same serotype as at least one of the alleles in the mixture of alleles.

17. A method according to claim 15 or 16 wherein the mixture of alleles is from a prospective recipient or a prospective donor in a tissue or organ transplant operation.

18. A method for determining whether a prospective recipient in a tissue or organ transplant operation has alleles of a gene that are compatible with the alleles of a prospective donor in the operation, which method comprises

(i) amplifying the alleles of the prospective recipient employing a pair of primers in which one of the primers carries a ligand, so as to produce amplified double-stranded alleles of the prospective recipient in which one of the strands carries a ligand;

(ii) contacting the amplified double-stranded alleles with a receptor on a solid support under conditions such that the ligand binds to the receptor;

(iii) separating the double-stranded alleles into single-strands and removing the strands that are not bound to the support by the ligand;

(iv) recovering the remaining strands from the support;

(v) mixing the recovered strands with complementary strands of the alleles of the prospective donor so as to form test duplexes;

(vi) separating the test duplexes by gel electrophoresis; and carrying out one or more of the following steps:

(vii) comparing the positions to which the test duplexe migrate on the gel with the position of a control DNA molecule;

(viii) sequencing the test duplexes;

(ix) sequence specific primer (SSP) amplification analysis; and

(x) sequence specific oligonucleotide (SSO) analysis.

19. A method according to claim 18 wherein the complementary strands of the alleles of the prospective donor are provided by

(i) amplifying the alleles of the prospective donor employing a pair of primers in which one of the primers carries a ligand, so as to produce amplified double-stranded alleles of the prospective donor in which one of the strands carries a ligand;

(ii) contacting the amplified double-stranded alleles with a receptor on a solid support under conditions such that the ligand binds to the receptor;

(iii) separating the double-stranded alleles into

single-strands and removing the strands that are not bound to the support by the ligand; and

(iv) recovering the remaining strands from the support.

20. A method according to claim 18 or 19 wherein the prospective donor is selected to have alleles of the same serotype as the prospective recipient.

21. A method according to any one of claims 18 to 20 wherein the control DNA is a homoduplex between two strands of the same allele and migration of the test duplexes to the same position on the gel as the homoduplex indicates that the prospective recipient and the prospective donor have the same alleles.

22. A method according to any one of claims 15 to 21 wherein the alleles are of a human leucocyte antigen (HLA) class I gene or an HLA class II gene.

23. A method according to claim 22 wherein the alleles are of the HLA-A gene, the HLA-B gene or the HLA-C gene.

24. A method according to any one of claims 2 to 4 and 11 to 23 wherein the ligand is biotin and the receptor is streptavidin.

25. A method according to any one of claims 2 to 4 and 11 to 24 wherein the solid support is magnetic beads, and the strands which do not carry a ligand are removed by attracting the beads to a magnet and washing the beads under conditions such that the double-stranded DNA molecules dissociate into single strands.

26. A method according to any one of the preceding claims wherein amplification of the DNA molecules is performed by

polymerase chain reaction (PCR).

27. A method according to any one of claims 17 to 26 wherein the prospective recipient and the prospective donor are a prospective bone marrow recipient and a prospective bone marrow donor, or a prospective kidney recipient and a prospective kidney donor.

28. A method according to any one of claims 1 to 27 wherein the DNA molecules in the mixture of DNA molecules have the same number of nucleotides but different base sequences.

29. A method for identifying a DNA molecule, which method comprises:

(i) contacting the DNA molecule with a labelled reference DNA strand under conditions such that the reference strand hybridizes to a complementary strand of the DNA molecule so as to form a test duplex;

(ii) running the test duplex and one or more control duplex(es) in a gel by electrophresis; and

(iii) comparing the position of the test duplex on the gel with the position(s) of the control duplex(es).

30. A method according to claim 29 wherein the control duplexes are either (a) duplexes which have faster and/or slower mobility than the test duplex and which are run in the same lane on the gel as the test duplex or (b) duplexes which have graded mobilities and which are run in a different lane on the gel to the test duplex.

31. A method according to claim 29 or 30 wherein the

reference strand is labelled with a fluorescent label or a label suitable for attachment of an enzyme.

32. A method according to any one of claims 29 to 31 wherein steps (i) to (iii) are repeated for a second or more times and a different reference strand is used in each repeat.

33. A method according to any one of claims 29 to 31 wherein steps (i) to (iii) are repeated a second time using a second reference strand.

34. A method according to claim 32 or 33 wherein the DNA molecule to be identified is an allele of a gene having from 10 to 300 alleles.

35. A method according to claim 34 wherein the gene has from 30 to 100 alleles.

36. A method according to any one of claims 29 to 35 wherein the gene is an HLA gene.

37. A method according to claim 36 wherein the gene is an HLA class I gene.

38. A method according to claim 37 wherein the gene is HLA-A, HLA-B or HLA-C.

ins B1